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the Her-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Overexpression and amplification of the Her-2/*neu* proto-oncogene has been implicated in the development of aggressive human breast cancer. Consequently, Her-2/neu provides a potential target for immunotherapy. Indeed, Her-2/neu specific cytotoxic T lymphocytes (CTL) can be detected in patients with breast and ovarian cancer. Nevertheless, the observed response to Her-2/*neu* is inadequate to prevent tumor progression. Our overall goal is to determine whether genetic vaccination is a feasible strategy to enhance Her-2/*neu* specific T cell activity, and in turn prevent and/or treat breast cancer. Specifically, we will employ a novel *in vivo* gene transfer system based on the Venezuelan Equine Encephalitis (VEE) virus. VEE is an alphavirus which has a number of properties that make it especially well suited for tumor antigen vaccination. The most salient of these is the observation that VEE preferentially infects dendritic cells (DC) *in vivo*. DC are known to be potent antigen presenting cells capable of efficiently activating naive $CD4^+$ and $CD8^+$ T cells. Our approach is to establish VEE based replicons encoding Her-2/*neu* as well as cytokines, such as IL-12 and IL-18 known to promote $CD4^+$ Th1 and $CD8^+$ CTL activity. To directly determine the therapeutic efficacy of the VEE-based replicons, mice transgenic for the rat Her-2/*neu* gene (FVB/*neu*) which develop spontaneous focal mammary tumors with subsequent pulmonary metastasis will be employed. Additionally, FVB/*neu* mice bred to mice transgenic for the human major histocompatibility complex (MHC) class I molecule HLA-A2.1 (FVB/*neu*/A2-K^b) will be used to examine the immunogenicity of Her-2/*neu* derived peptides which may have clinical relevance. We believe establishing a vaccine protocol using VEE replicons encoding Her-2/*neu* and appropriate cytokines will provide an effective strategy for enhancing tumor antigen-specific $CD4^+$ Th1 and $CD8^+$ CTL reactivity for the purpose of preventing tumor progression and providing long-term protection from tumor recurrence.

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Overview:

During the past year, we have been focusing on the objectives outlined for Specific Aim 2 in our approved statement of work, and on completing the objectives for Specific Aim 1. In addition to having completed testing and packaging of VEE replicon constructs encoding Her-2/neu, IL-12, IL-18, IL-2 and mGM-CSF as reported previously, we have completed *in vitro* testing and packaging of a truncated Her-2/neu replicon that encodes only the extracellular and transmembrane domains of the Her-2/neu protein (Neu/ECD-TM). We have begun a series of *in vivo* experiments to evaluate the therapeutic efficacy of this replicon and have obtained our most encouraging results to date.

We have begun a series of experiments in both wild type FVB, and in FVB mice transgenic for the rat Her-2/*neu* gene (FVB/*neu*) in which both sets of mice are vaccinated with VEE replicons and subsequently challenged with Her-2/*neu* expressing tumors. Specifically, both wild type mice which reject the tumor cells, and transgenic mice, which are normally permissive for growth, were twice immunized with 5.0×10^5 VEE replicons encoding Neu/ECD-TM ten days apart. Mice were subsequently challenged with 2.0×10^6 Her-2/*neu*$^+$ F-H2N1 tumor cells injected subcutaneously in the rear flank. Immunized transgenic mice displayed a significant delay in the appearance of tumor formation with one mouse remaining tumor free throughout the course of the experiment (Figure 1). In contrast, unimmunized FVB/*neu* mice rapidly develop palpable tumors within 10 – 14 days following injection. As expected, both untreated and immunized wild type FVB mice rejected the tumors in all but one instance. In addition to repeating this experiment, we have immunized several groups of transgenic mice with the intention of monitoring spontaneous tumor formation compared to untreated controls.

The rat Her-2/*neu* gene encodes for an 185kDa transmembrane protein. In order that we may have protein to work with experimentally, we have spliced fragments of the gene into a bacterial expression plasmid that generates HIS tagged protein fragments which may be purified on nickel resin. One protein generated from the bacterial expression system, encompassing parts of the extracellular and transmembrane domains, has been used to stimulate $CD4^+$ T cell proliferation. Currently, we are working on a protocol in which the HIS tagged protein fragments are affixed to nickel chelate beads. Aggregation of the protein fragments in this manner allows for enhanced uptake by antigen presenting cells and subsequent peptide loading into the MHC Class I pathway for presentation to $CD8^+$ T cells[1]. We are hopeful that this system for loading class I restricted peptides on APCs will expand our ability to characterize the $CD8^+$ T cells response to Her-2/neu encoding replicons through the use of *in vivo* CTL and ELISPOT assays.

Recent reports have demonstrated the utility of *in vivo* CTL assays using Carboxyfluorescein diacetate succinimidyl ester (CFSE) labeled target cells[2]. Briefly, splenocytes are pulsed with peptide (to which the subject has been immunized) and labeled with 5μm CFSE. As an internal control, non-pulsed splenocytes are labeled with 0.5 μm CFSE, admixed and adoptively transferred to immunized mice. Upon analysis of recovered cells by flow cytometry, the disappearance of antigen pulsed cells (CFSE high peak) relative to internal control cells (CFSE low peak) is indicative of CTL activity. While we have established this system in the lab in another transgenic murine system, the lack of a H2-K^d restricted Her-2/*neu* peptide has hampered our efforts in this system. Consequently, the above mentioned nickel bead chelation protocol will allow us to label target cells and more effectively evaluate CTL activity in Her-2/*neu* immunized mice.

As we have completed testing and packaging of several cytokine encoding replicons, we plan to expand our immunization protocol to include these constructs. We believe the expression of proinflammatory cytokines will further expand the antigen specific population and increase CTL activity. We are working toward determining the optimal combinations of antigen and cytokine encoding VEE replicons for efficient generation of $CD4^+$ Th and $CD8^+$ CTL reactivity. This will further allow us to establish an effective approach of immunotherapy targeting Her-2/*neu* for the treatment end prevention of breast adenocarcinoma in transgenic mice.

Key Accomplishments:

- Completed in vitro testing and packaging of the truncated Her-2/*neu* VEE replicon encoding the extracellular and transmembrane domains of the protein.
- Completed two tumor challenge experiments in VEE Her-2/*neu* (ECD-TM) replicon immunized FVB/*neu* mice demonstrating significantly delayed tumor development.
- We have begun experiments to evaluate the efficacy of VEE replicons encoding the extracellular and transmembrane domains of Her-2/*neu* in the prevention of spontaneous breast adenocarcinoma development in FVB/*neu* mice.
- We are in the process of modifying an in vivo CTL assay protocol for use in the Her-2/neu transgenic murine model.

Reportable Outcomes:

- None.

Figures:

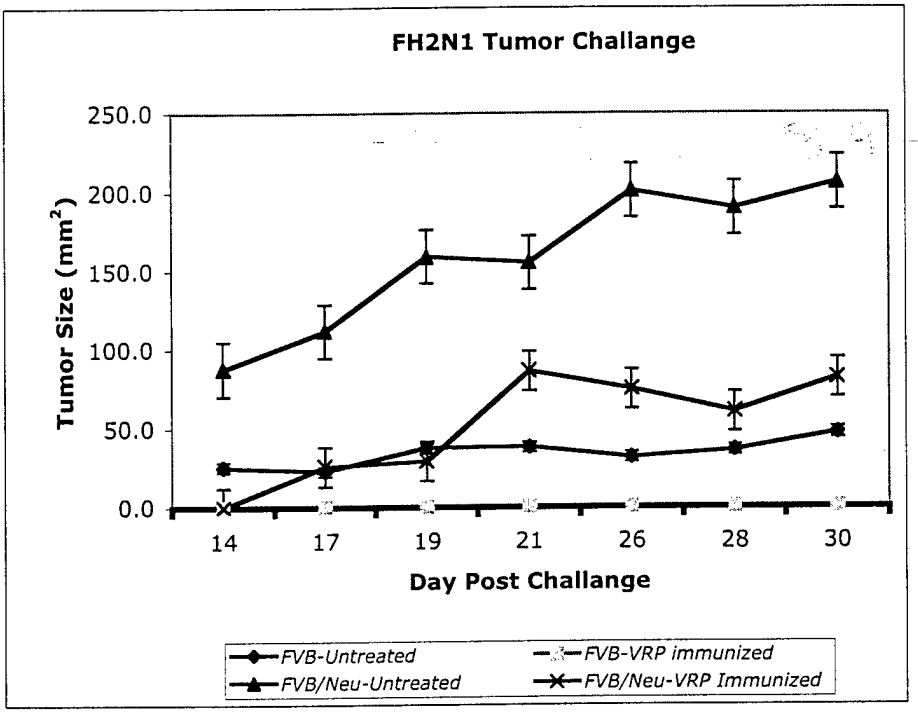

Figure 1. Delayed tumor growth in VEE replicon immunized mice. FVB and FVB/*neu* mice were immunized twice at 10-day intervals with 5.0 x 10^5 IU VEE replicons encoding the extracellular and transmembrane domains of Her-2/*neu*. One week after the second vaccination, mice received 2.0 x 10^6 F-H2N1 tumor cells injected subcutaneously in the left flank. Challenged mice were monitored for tumor growth over a 30-day period and palpable tumors were measured using a Vernier type caliper. Data is representative of two different experiments, four mice per group. One immunized FVB/*neu* mouse remained tumor free throughout the experiment.

Summary:

We have completed much of the work described for Specific Aim 1, and have made significant progress on the experiments required for Specific Aim 2. We have completed all in vitro testing and packaging of replicons including the truncated Her-2/*neu* replicon encoding the extracellular and transmembrane domains of the protein. We have made progress in evaluating the therapeutic efficacy of VEE replicons in preventing the development and progression of breast adenocarcinoma in mice challenged with F-H2N1 tumor cells. Specifically, we have demonstrated delayed onset and inhibited tumor growth in challenged mice immunized with the truncated Her-2/*neu* replicon. We have begun a series of experiments to evaluate the efficacy of VEE replicons in preventing the spontaneous development of breast adenocarcinoma in FVB/neu mice.

While we have been able to consistently demonstrate CTL activity against Her-2/neu in immunized FVB mice, obtaining these same results in FVB/*neu* mice has proved challenging. We have begun experimenting with a new, more sensitive system for evaluating CTL activity in vivo using antigen pulsed target cell labeled with CFSE. We are hopeful that this assay will allow us to correlate CTL activity with inhibited tumor progression in immunized FVB/neu mice. Additionally, we are working on a protocol for coupling HIS tagged Her-2/*neu* protein fragments to nickel beads. These beads should prove useful for labeling APCs with antigen for use with *in vivo* CTL assays as well as ELISPOT assays to further characterize the $CD4^+$ and $CD8^+$ T cell cytokine secretion profile.

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